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## Article

# Tree Species Identity Shapes Earthworm Communities

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**Abstract:** Earthworms are key organisms in forest ecosystems because they incorporate organic material into the soil and affect the activity of other soil organisms. Here, we investigated how tree species affect earthworm communities via litter and soil characteristics. In a 36-year old common garden experiment, replicated six times over Denmark, six tree species were planted in blocks: sycamore maple (*Acer pseudoplatanus*), beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*), Norway spruce (*Picea abies*), pedunculate oak (*Quercus robur*) and lime (*Tilia cordata*). We studied the chemical characteristics of soil and foliar litter, and determined the forest floor turnover rate and the density and biomass of the earthworm species occurring in the stands. Tree species significantly affected earthworm communities via leaf litter and/or soil characteristics. Anecic earthworms were abundant under *Fraxinus*, *Acer* and *Tilia*, which is related to calcium-rich litter and low soil acidification. Epigeic earthworms were indifferent to calcium content in leaf litter and were shown to be mainly related to soil moisture content and litter C:P ratios. Almost no earthworms were found in *Picea* stands, likely because of the combined effects of recalcitrant litter, low pH and low soil moisture content.

**Keywords:** biogeochemistry; litter quality; soil fauna; soil acidification; plant–soil interactions; biological indicator of soil quality; Oligochaeta

## 1. Introduction

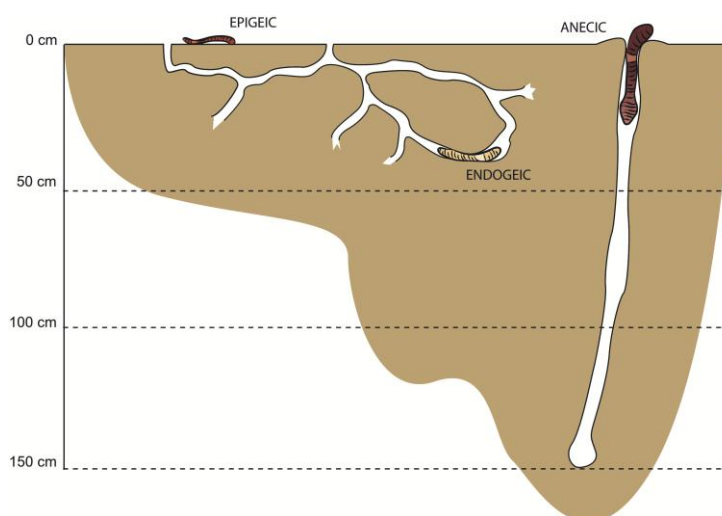
Earthworms have been studied for a long time (e.g., Darwin [1]), and it is known that soil and forest floor characteristics profoundly affect the composition and abundance of earthworm populations [2–4]. On the other hand, earthworms are ecosystem engineers [5,6] that can physically, chemically and biologically modify their environment, impacting the habitat and the resources for other organisms [7–9] and consequently providing a wide diversity of ecosystem services including facilitation of nutrient cycling and formation of stable humic compounds [6] and mineral soil C sequestration [10,11].

Earthworm species all have distinct feeding niches varying from fresh leaf litter to humus and even animal dung, fungal hyphae or soil [12]. The simultaneous occurrence of species from different ecological groups can have synergistic outcomes, and loss of species can lead to significant changes in the ecosystem services they provide [13].

Earthworm species can be classified according to their morphological features, habitat choice and feeding habits into three ecological groups: epigeic, endogeic and anecic earthworms (Figure 1, [14]). First, epigeic earthworms, or ‘litter-dwellers’, are detritivorous species feeding mainly on fresh or partially decomposed litter on or near the soil surface. They contribute to litter fragmentation, but have too little muscular power to enter the mineral soil. Given the important organic acid production in accumulating forest floors, most epigeic species are tolerant to acid conditions [15]. For example, *Dendrobaena octaedra* can occur in *Pinus*, *Picea* or *Fagus* stands with soil  $\text{pH}_{\text{H}_2\text{O}}$  of less than 4.0 [16,17].

Secondly, endogeic earthworms, or ‘soil-dwellers’, are geophagous earthworms feeding on largely humified soil organic matter and dead roots. They are literally eating their way through the shallow soil and, thereby, ingesting large quantities of soil and mixing this with organic material, i.e., bioturbation [12]. Most of these species are very sensitive to soil acidification [15,17], for example, the fitness of *Aporrectodea caliginosa* declined in acid soils ( $\text{pH}_{\text{H}_2\text{O}} < 4.8$ , [18]).

Thirdly, anecic earthworms, or ‘deep-burrowers’, are also detritivorous but differ from epigeic earthworms by their ability to create deep vertical burrows in the mineral soil. They can pull leaf litter from the soil surface into their burrows. The anecic species *Lumbricus terrestris* is known for removing significant quantities of litter from the forest floor [12] while casts are mainly deposited at the soil surface [19]. This species can occur in a broad pH-range ( $\text{pH}_{\text{H}_2\text{O}}$  4.0–7.2 [17,20]) of soil acidity but main occurrence of the anecic species *Aporrectodea longa* and *Lumbricus terrestris* is at  $\text{pH}_{\text{H}_2\text{O}}$  above 4.6 [21].



**Figure 1.** Illustration of the three ecological earthworm groups according to their food preference and behavior. The brown zone represents the soil. Reproduced from [22].

In forests, the environmental factors that regulate earthworm communities include litter traits and soil characteristics, mainly clay content, pH, base saturation, soil moisture content, organic matter content and aluminium (Al) toxicity [2,23]. Soil pH correlates strongly positively with soil calcium (Ca) and negatively with soil Al concentration, and is among the most important drivers for anecic and endogeic earthworms, i.e., the burrowing earthworm community. A study in multiple *Fagus sylvatica* stands on a gradient from acid to limestone soils showed that burrowing earthworm densities were most strongly positively linked with the presence of a limestone layer and almost absent from Ca-poor soils [24].

Tree species can significantly modify most of these soil characteristics [25–28]. For instance, N<sub>2</sub>-fixing tree species as *Alnus glutinosa* with high nitrification rates can acidify the topsoil, which hampers the activity of burrowing earthworms despite the high nutrient content of alder litter [27]. It is known that the quality of litter as a substrate for earthworms increases with N, P, K, Ca, and Mg

concentrations and decreases with increasing lignin, lignin:N, and C:N ratios [29]. Tree species can reinforce patterns of soil fertility through positive and/or negative litter feedbacks on earthworm activity and the rate of nutrient cycling [30]. For example, several common garden experiments have shown that in just a few decades, tree species with slowly decomposing litter of poor quality for earthworms as *Picea abies*, *Fagus sylvatica* and *Quercus robur* acidify soils and create humus forms very different from those under species with fast decomposing nutrient-rich litter such as *Fraxinus excelsior*, *Acer pseudoplatanus* and *Tilia cordata* [27,31,32]. The latter tree species thus lead to a higher earthworm species diversity and total earthworm biomass (4–6 species, 11–37 g·m<sup>-2</sup>) compared to species with more nutrient-poor litter (1–2 species, 0–4 g·m<sup>-2</sup> [33]).

Epigeic and anecic earthworm species seem to be mainly affected by litter traits while endogeic earthworm species are affected by soil characteristics [23]. However, it seems that these patterns depend on the soil context. For example, it was shown in acidic soils that endogeic earthworms were also significantly linked with several litter quality characteristics such as N, Mg and Ca concentration [34].

Here we investigate the effect of six common European tree species with diverging litter quality on earthworm communities. We used a 36-year-old common garden experiment with sites distributed across Denmark to verify how the identity of tree species results in very different earthworm community assemblages. This replicated common garden experiment has previously revealed tree species effects on C and N stocks in the forest floor and mineral soil [35], soil respiration and soil organic C turnover [36] and on N cycling and leaching and the water budget [37]. We aim to broaden the understanding of plant–soil interactions in this common garden experiment by combining existing data on various litter quality characteristics and forest floor turnover rate with new data on soil biogeochemistry and earthworm communities. We expected the tree species to also affect the earthworm communities via altered soil and litter quality characteristics. We also assumed the need to look beyond the response of rough earthworm ecological categories, and rather focus on species-specific responses to varying litter and soil characteristics. We further discuss how our results can be used in the practice of forestry and in light of ongoing global changes.

## 2. Materials and Methods

### 2.1. Common Garden

This study was carried out in a 36-year-old common garden experiment replicated in six sites in Denmark (Appendix A). Two out of six study sites (Kragelund and Matstrup) were former agricultural land while the other sites were previously forested with *Fagus sylvatica* since the beginning of the 19<sup>th</sup> century. Each site was planted with 0.25 ha adjacent unreplicated monoculture stands of six common European tree species: *Acer pseudoplatanus* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Picea abies* (L.) H. Karst., *Quercus robur* L. and *Tilia cordata* Mill., except for Vallø, where the *Fraxinus* stand establishment failed due to deer browsing. For further details on the setup of this common garden experiment, we refer to Table 1 in [35].

### 2.2. Soil Sampling and Analyses

In each stand, we randomly selected three representative plots of 0.25 m<sup>2</sup> (total number of plots = 105) where we assessed earthworm density and biomass in October 2009 with a minimum distance of 10 m from the stand border. In each plot, three soil cores were combined into one composite soil sample for each of three depths below the forest floor (0–0.05 m, 0.05–0.15 m and 0.15–0.3 m). Soil moisture content was measured gravimetrically by the weight difference before and after drying to constant weight at 40 °C. After sieving (2 mm sieve), soil pH-KCl was determined in a 1:5 soil/KCl solution (1 M) with a glass electrode (Ross Sure-flow 8172). The exchangeable concentrations of K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup> and Al<sup>3+</sup> were measured by flame atomic absorption spectrophotometry using BaCl<sub>2</sub> (0.1 M) as extractant (ISO 11260). Effective cation exchange capacity (CEC) was calculated as the sum of K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup> and Al<sup>3+</sup>, expressed in meq·kg<sup>-1</sup>. The effective base saturation (BS) was calculated by dividing the sum of the base cations (K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup>) by the CEC.

### 2.3. Sampling and Analyses of Litter and Forest Floor

Litterfall and forest floor sampling was described in detail in Vesterdal et al. [35] and Vesterdal et al. [36]. Briefly, litterfall was collected monthly using ten circular littertraps with a diameter of 31 cm installed along two line transects. Litterfall was sampled in all species for one full year at two sites (Matstrup and Vallø) and at the remaining four sites the broadleaf species litterfall was sampled in the autumn to gauge the discrete foliar litterfall event. Annual litterfall amounts for the broadleaves were estimated by proportional upscaling using the annual litterfall amounts measured at Matstrup and Vallø. Litterfall was dried at 55 °C and hand-sorted and weighed in two fractions: foliar and non-foliar litter. Forest floor was sampled in 15 points along three line transects within each stand using a 25 cm × 25 cm wooden frame in September 2004 just before the onset of foliar litterfall for deciduous species, when forest floor mass was at a minimum. Foliar fractions of the forest floor were dried to constant weight at 55 °C before weighing. In the further analyses, we have used one value per stand for these litter and forest floor traits ( $n = 35$ ).

### 2.4. Earthworm Sampling and Identification

In each plot, we used a combined sampling method, ensuring effective sampling of different ecological groups of earthworm species. First, the litter-dwelling specimen was collected by hand-sorting of a litter sample within a 0.25 m<sup>2</sup> frame; then, the deep-burrowing specimen was captured at the soil surface by application of a mustard solution (60 g mustard powder in 30 L water, [38,39]) within the same 0.25 m<sup>2</sup> frame, and 30 min after mustard extraction, the surface soil dwelling specimen was collected by hand-sorting of a soil core taken in the center of the frame (0.09 m<sup>2</sup> with a 0.2 m depth). The earthworms were, for each of the three methods separately, collected in pots containing ethanol (95%) and after a few hours transferred to a 5% formalin solution for fixation. After three days, they were transferred back to a 95% ethanol solution for further preservation and identification. All adult earthworms were identified with the key of Sims and Gerard [15] and were subsequently categorized into three ecological groups as defined by Bouché [40] (epigeic, endogeic or anecic species). In case of individuals with missing heads or juveniles, identification at species level was not possible. We identified these individuals to genus or ecological group level and assigned them pro rata to species. There are disadvantages to this prorating method [41] but since only about half of the individuals per plot were adult, it was important to include these juvenile and unidentifiable individuals. All worms were weighed individually, including gut contents, after briefly being dried on filter paper at room temperature. We did not correct earthworm biomass for potential bias caused by the gut contents. The density and biomass of earthworms sampled by the hand-sorting method was converted to an area of 0.25 m<sup>2</sup>. Then, for each of the plots, we calculated the area-weighted sum of the number and biomass of earthworms from the three sampling methods per m<sup>2</sup>. The number of earthworms per m<sup>2</sup> will hereafter be referred to as the earthworm density.

### 2.5. Calculations

#### 2.5.1. Handling of Missing Data

The foliar litterfall data for *Picea* stands in Kragelund, Odsherred, Viemose and Wedellsborg was missing. Instead, we used the mean values for the mass, C, N, P, Ca, Mn, lignin from Matstrup and Vallø based on evidence of limited site effects on litterfall mass across Denmark [42].

#### 2.5.2. Forest Floor Turnover Rate

The forest floor turnover rate ( $k$ ) for the broadleaved tree species was calculated according to the following equation for discrete litterfall events [43] because these tree species mainly shed their litter from September to November [35,42]:

$$k = \frac{DM_{Litterfall}}{DM_{Litterfall} + DM_{Forest\ floor}}$$

where  $k$  is the annual decay rate;  $DM_{Litterfall}$  is the average annual foliar litterfall measured, and  $DM_{Forest\ floor}$  is the accumulated foliar litter layer on the forest floor.

For *Picea*, a tree species with continuous litterfall through the year [42],  $k$  was calculated by a different equation [43]:

$$k = \frac{DM_{Litterfall}}{DM_{Forest\ floor}}$$

## 2.6. Data Analyses

All statistical analyses were performed with R [44].

### 2.6.1. Tree Species Effect

The effect of tree species identity on the litter ( $n = 35$ ) and soil variables ( $n = 105$ ) was analyzed using linear multilevel models with site as a group-level effect to account for the spatial dependence of the measurements (e.g., in R syntax `response variable~Treespecies-1, random= ~1|Site; lme;` package `nlme` [45]). Differences between tree species were tested with Tukey post-hoc tests ( $p < 0.05$ ) using the function `glht` from the `multcomp` package [46]. Model fit was verified by examination of the residuals; we log-transformed the response variables in case it improved the fit ('soil K', 'soil Na', 'soil Mg', 'soil Ca', 'soil Al', 'litter Mn' and 'forest floor mass').

Due to the high frequency of zero's in the earthworm response variables (25%–35% for ecological earthworm groups and more for individual species), we used generalized linear multilevel models that allowed for zero-inflation (`glmmADMB`; package `glmmADMB` [47]). A negative binomial response distribution was assumed to allow for overdispersed earthworm count data [48–50]. Model syntax was similar and differences between tree species were also tested with Tukey post-hoc tests in the method that was described previously.

### 2.6.2. Links between Litter Quality, Soil Quality and Earthworms

To explore the particular soil and litter variables underlying the overall tree species effect on earthworms ( $n = 105$ , models above), in models explaining various earthworm responses (the number of species, total, epigeic, endogeic and anecic earthworm density and biomass) we performed model selection with several soil and litter variables as predictors and site as a group-level effect (again `glmmADMB` allowing for zero-inflation). The included predictors, standardized by centering to the mean and rescaling by their standard deviation, were 'soil moisture content, soil pH 0–5 cm, soil Al 0–5 cm, soil Na 0–5 cm, litter Ca, litter N, litter Mn, litter P, litter lignin, litter C:N ratio, litter C:P ratio, forest floor turnover rate and the interaction between soil Al 0–5 cm and litter Ca'. We have also included a measure for the possible interaction between litter quality and soil quality affecting earthworm communities. Leaf litter Ca concentration appears to be an important litter quality trait affecting earthworms [26,27,31]. Further, pH is an important soil quality trait for earthworm activity [23]. Since exchangeable Al concentrations are strongly linked with pH-KCl and probably affect earthworm activity (indications of its toxicity for earthworms in [51]) we also included exchangeable Al to our models. Because exchangeable Al concentration seemed to be more important than pH-KCl in our models, we selected exchangeable Al concentration as a measure for soil quality in the interaction (Soil-Al:Litter-Ca).

To check for multicollinearity, we calculated the variance inflation factors of the standardized variables in a full model. None of the variables had a variance inflation factor (VIF)  $> 3$  and therefore, we could include these terms in our model selection [52].

We performed manual stepwise forward selection of predictor terms starting with null models where only site as a group-level effect was included. We added each of the predictors, calculated the Akaike information criterion corrected for small sample sizes ( $AIC_c$ ) [53] using `AICctab` (package `bbmle`) and continued with the model with the smallest  $AIC_c$  value.  $AIC_c$  values,  $\Delta AIC_c$  values and Akaike weights of the null, intermediate and final optimal models are shown in Appendix B. Predictor variables were added until the  $AIC_c$  was minimized and, then, we evaluated the

significance of the included variables by the Wald test ( $p < 0.05$ ). If these models contained only significant variables, they were retained as the final optimal models. If these models contained non-significant variables, we went back one step in the selection process. We assessed goodness of fit of the optimal models by calculating a measure for  $R^2$  describing the correlation between fitted and observed values by the *r2.corr.mer* function, and we further evaluated plots of residuals and fitted versus observed data. We plotted the coefficients from these models with *coefplot2* (*coefplot2* package).

### 3. Results

#### 3.1. The Tree Species Effect on Soil Properties and Litter Quality

After 36 years of forestation, tree species consistently influenced all measured topsoil properties significantly across the six sites (Table 1). According to the pH (0–5 cm), the tree species could be ranked as: *Fraxinus* = *Acer* = *Tilia* >> *Quercus* = *Fagus* >> *Picea*. *Picea* had significantly lower soil moisture content, pH, exchangeable base cation concentrations and higher Al and Na concentrations. The tree species affected not only the topsoil (0–5 cm), as *Picea* stands also had significantly increased Na and Al concentrations in the deeper soil layers (5–15 cm and 15–30 cm; Appendix C) compared to the other tree species.

**Table 1.** Mean and standard deviation of topsoil (0–5 cm) properties for each tree species across all six common gardens. Significant differences between tree species are indicated with letters, means with the same letter are not significantly different (Tukey post-hoc tests on linear mixed-effects (LME) models, 1|Site).

Soil variables (0–5 cm)	<i>f</i> -value	<i>p</i>	Tree Species					
			<i>Fraxinus</i>	<i>Acer</i>	<i>Tilia</i>	<i>Quercus</i>	<i>Fagus</i>	<i>Picea</i>
Moisture (%)	1475	<0.001	14 ± 5 <sup>c</sup>	15 ± 4 <sup>c</sup>	12 ± 3 <sup>b</sup>	13 ± 4 <sup>bc</sup>	12 ± 4 <sup>b</sup>	9 ± 2 <sup>a</sup>
pH-KCl	325	<0.001	4.2 ± 0.6 <sup>c</sup>	4.2 ± 0.5 <sup>c</sup>	4.0 ± 0.4 <sup>c</sup>	3.7 ± 0.3 <sup>b</sup>	3.7 ± 0.2 <sup>b</sup>	3.5 ± 0.2 <sup>a</sup>
Base saturation (%)	108	<0.001	73 ± 28 <sup>b</sup>	78 ± 24 <sup>b</sup>	71 ± 20 <sup>b</sup>	49 ± 20 <sup>a</sup>	49 ± 21 <sup>a</sup>	41 ± 19 <sup>a</sup>
K <sup>+</sup> in BaCl <sub>2</sub> (mg·g <sup>−1</sup> )	50	<0.001	100 ± 88 <sup>bc</sup>	114 ± 91 <sup>c</sup>	91 ± 56 <sup>bc</sup>	85 ± 57 <sup>bc</sup>	67 ± 42 <sup>ab</sup>	41 ± 22 <sup>a</sup>
Na <sup>+</sup> in BaCl <sub>2</sub> (mg·g <sup>−1</sup> )	28	<0.001	19 ± 16 <sup>a</sup>	17 ± 11 <sup>a</sup>	15 ± 8 <sup>a</sup>	13 ± 7 <sup>a</sup>	13 ± 7 <sup>a</sup>	38 ± 48 <sup>b</sup>
Mg <sup>2+</sup> in BaCl <sub>2</sub> (mg·g <sup>−1</sup> )	48	<0.001	139 ± 106 <sup>c</sup>	108 ± 72 <sup>bc</sup>	81 ± 39 <sup>ab</sup>	68 ± 53 <sup>a</sup>	49 ± 32 <sup>a</sup>	57 ± 41 <sup>a</sup>
Ca <sup>2+</sup> in BaCl <sub>2</sub> (mg·g <sup>−1</sup> )	42	<0.001	1241 ± 1020 <sup>c</sup>	1050 ± 690 <sup>bc</sup>	796 ± 437 <sup>ab</sup>	481 ± 388 <sup>a</sup>	446 ± 293 <sup>a</sup>	467 ± 351 <sup>a</sup>
Al <sup>3+</sup> in BaCl <sub>2</sub> (mg·g <sup>−1</sup> )	42	<0.001	115 ± 121 <sup>a</sup>	87 ± 58 <sup>a</sup>	151 ± 118 <sup>a</sup>	261 ± 121 <sup>bc</sup>	231 ± 105 <sup>b</sup>	309 ± 133 <sup>c</sup>

All measured foliar litter property variables were significantly influenced by the tree species (Table 2). Ca, Mg and K concentrations in leaf litter of *Fraxinus* and *Acer* were at least double the concentrations in leaf litter of *Fagus* and *Picea*. Lignin leaf litter concentrations were significantly higher in *Picea*, *Tilia*, *Quercus* and *Fagus* (ranging from 25% to 29%) compared to *Fraxinus* and *Acer* (on average 18%). Leaf litter of *Fraxinus* and *Acer* had significantly lower C:N ratios compared to *Quercus*, *Picea* and *Fagus*. Also, Mn and P in leaf litter varied significantly across tree species, with *Fraxinus* containing significantly lower Mn and higher P concentrations compared to the other species.

Also, the forest floor masses and turnover rates were significantly differing between the observed tree species, with *Picea* having the lowest forest floor turnover rate and the highest forest floor mass, and *Fraxinus*, *Acer* and *Tilia* stands having the highest forest floor turnover rates and lowest forest floor masses. *Quercus* and *Fagus* were found to be intermediate.

**Table 2.** Mean and standard deviation of annual litterfall, foliar litter quality and forest floor accumulation for the tree species across all six common gardens. Significant differences between tree species are indicated with letters, means with the same letter are not significantly different (Tukey post-hoc tests on LME models, 1|Site). Litterfall and forest floor foliar mass and nutrient concentrations in litter were previously published by Vesterdal et al. [35,36].

	<i>f</i> -value	<i>p</i>	Tree Species					
			<i>Fraxinus</i>	<i>Acer</i>	<i>Tilia</i>	<i>Quercus</i>	<i>Fagus</i>	<i>Picea</i>
<i>Litterfall</i>								
Foliar mass (Mg·ha <sup>-1</sup> ·year <sup>-1</sup> )	127	<0.001	2.7 ± 1.0 <sup>bc</sup>	2.8 ± 0.39 <sup>c</sup>	2.4 ± 0.68 <sup>ab</sup>	2.6 ± 0.55 <sup>abc</sup>	2.1 ± 0.34 <sup>a</sup>	3.9 ± 0.46 <sup>d</sup>
C:N ratio	151	<0.001	25 ± 5.4 <sup>a</sup>	27 ± 2.6 <sup>ab</sup>	28 ± 3.5 <sup>ab</sup>	32 ± 3.0 <sup>b</sup>	42 ± 14 <sup>d</sup>	36 ± 1.9 <sup>c</sup>
C:P ratio	5200	<0.001	358 ± 128 <sup>a</sup>	477 ± 81 <sup>b</sup>	407 ± 87 <sup>a</sup>	415 ± 44 <sup>a</sup>	575 ± 113 <sup>c</sup>	473 ± 37 <sup>b</sup>
N (mg·g <sup>-1</sup> )	250	<0.001	19 ± 3.6 <sup>d</sup>	17 ± 1.7 <sup>c</sup>	18 ± 2.5 <sup>c</sup>	16 ± 1.5 <sup>b</sup>	13 ± 3.6 <sup>a</sup>	13 ± 0.74 <sup>a</sup>
Ca (mg·g <sup>-1</sup> )	124	<0.001	21 ± 5.3 <sup>c</sup>	19 ± 4.0 <sup>c</sup>	17 ± 4.3 <sup>b</sup>	10 ± 1.7 <sup>a</sup>	11 ± 1.8 <sup>a</sup>	10 ± 0.03 <sup>a</sup>
Mn (mg·g <sup>-1</sup> )	1025	<0.001	0.23 ± 0.27 <sup>a</sup>	0.63 ± 0.33 <sup>b</sup>	1.1 ± 0.52 <sup>c</sup>	1.6 ± 0.50 <sup>d</sup>	1.6 ± 0.55 <sup>d</sup>	1.2 ± 0.04 <sup>c</sup>
P (mg·g <sup>-1</sup> )	362	<0.001	1.5 ± 0.56 <sup>d</sup>	1.0 ± 0.19 <sup>ab</sup>	1.3 ± 0.28 <sup>cd</sup>	1.2 ± 0.13 <sup>bc</sup>	0.87 ± 0.18 <sup>a</sup>	1.0 ± 0.08 <sup>ab</sup>
Lignin (%)	506	<0.001	18 ± 2.8 <sup>a</sup>	18 ± 3.3 <sup>a</sup>	27 ± 4.1 <sup>c</sup>	27 ± 2.6 <sup>bc</sup>	29 ± 1.6 <sup>d</sup>	25 ± 0.17 <sup>b</sup>
<i>Forest floor</i>								
Foliar mass (Mg·ha <sup>-1</sup> )	39	<0.001	0.57 ± 0.4 <sup>a</sup>	1.7 ± 1.4 <sup>a</sup>	1.7 ± 1.0 <sup>a</sup>	6.0 ± 2.6 <sup>b</sup>	8.1 ± 2.8 <sup>b</sup>	37 ± 10 <sup>c</sup>
Foliar forest floor turnover rate (year <sup>-1</sup> )	93	<0.001	0.80 ± 0.18 <sup>e</sup>	0.68 ± 0.18 <sup>de</sup>	0.60 ± 0.20 <sup>d</sup>	0.35 ± 0.17 <sup>c</sup>	0.23 ± 0.09 <sup>b</sup>	0.11 ± 0.02 <sup>a</sup>

### 3.2. Tree Species Effect on Earthworm Populations

Over all stands and sites, we found 12 earthworm species (Table 3). Earthworm species richness in *Picea* plots (2 ± 2 species m<sup>-2</sup>) was significantly lower compared to *Tilia* (5 ± 1 species m<sup>-2</sup>), *Fraxinus* (4 ± 2 species m<sup>-2</sup>), *Acer* (4 ± 1 species m<sup>-2</sup>) and *Quercus* plots (4 ± 3 species m<sup>-2</sup>), while *Fagus* plots (3 ± 2 species m<sup>-2</sup>) were not significantly distinguished from the former tree species.

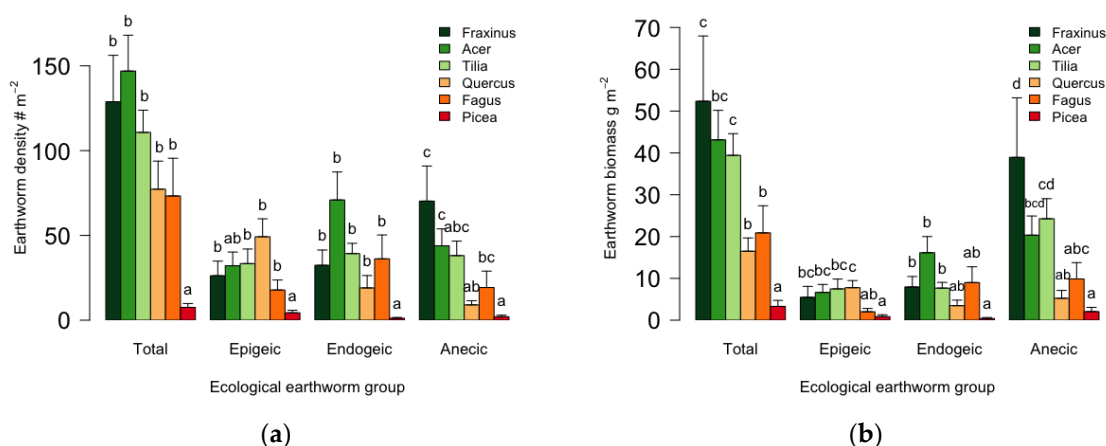
**Table 3.** Collected earthworm species, their ecological group according to Sims and Gerard [15], their incidence at the studied sites (K = Kragelund, M = Matrup, O = Odsherred, V = Vallø, Vi = Viemose, W = Wedellsborg) and tree species (Fr = *Fraxinus*, Ac = *Acer*, Ti = *Tilia*, Qu = *Quercus*, Fa = *Fagus*, Pi = *Picea*).

Earthworm Species	Ecological Group	Sites	Tree Species
<i>Allolobophora eiseni</i> (Levinsen)	Epigeic	K	Pi
<i>Dendrodrilus rubidus</i> (Savigny)	Epigeic	K M O V W	Fr Ac Ti Qu Fa Pi
<i>Dendrobaena octaedra</i> (Savigny)	Epigeic	K M O V Vi W	Fr Ac Ti Qu Fa Pi
<i>Eisenia fetida</i> (Savigny)	Epigeic	Vi	Ac
<i>Lumbricus castaneus</i> (Savigny)	Epigeic	K M O V	Fr Ac Ti Qu Fa
<i>Lumbricus festivus</i> (Savigny)	Epigeic	W	Ac Ti Qu
<i>Lumbricus rubellus</i> (Hoffmeister)	Epigeic	K M O V W	Fr Ac Ti Qu Fa Pi
<i>Lumbricus terrestris</i> (Linnaeus)	Anecic	K M O V Vi W	Fr Ac Ti Qu Fa Pi
<i>Aporrectodea longa</i> (Ude)	Anecic	M W	Fr Ac Ti Qu Fa Pi
<i>Aporrectodea caliginosa</i> (Savigny)	Endogeic	K M O V Vi W	Fr Ac Ti Qu Fa Pi
<i>Aporrectodea rosea</i> (Savigny)	Endogeic	M O V Vi W	Fr Ac Ti Qu Fa Pi
<i>Octolasion cyaneum</i> (Savigny)	Endogeic	O Vi W	Fr Ac Ti Qu Fa

Total earthworm density in the studied plots ranged from zero to 428 individuals m<sup>-2</sup> and total earthworm biomass amounted to maximally 202 g·m<sup>-2</sup>. Figure 2 shows the tree species-specific responses in earthworm communities. In *Picea* stands, earthworm populations were absent from half of the plots, and across all sites, significantly, they contained the lowest total earthworm densities and biomasses of all tree species. *Quercus* and *Fagus* stands contained, on average, an intermediate total earthworm density and biomass. The highest earthworm densities and biomasses were found in *Fraxinus* and *Acer* stands and *Fraxinus* and *Tilia* stands, respectively.

Considering the three ecological groups, we found *Fraxinus*, *Acer* and *Tilia* stands to have higher densities and biomasses than *Picea* stands for all ecological groups (Figure 2). Epigeic earthworm biomass was significantly higher in *Quercus* stands compared to *Picea* and *Fagus* stands. Density and biomass of endogeic earthworms were significantly higher in *Acer* stands than in *Picea* stands, while anecic earthworm density and biomass were significantly higher in *Fraxinus* stands compared to *Quercus*, *Fagus* and *Picea* stands.





**Figure 2.** Density (a) and biomass (b) of total, epigeic, endogeic and anecic earthworms (mean + standard error). The tree species were sorted according to decreasing soil pH-KCl (0–5 cm). Significant differences between tree species within each earthworm group are indicated with letters (Tukey post-hoc tests on generalized linear multilevel (glmmADMB) models, 1 | Site).

### 3.3. Links between Soil Quality, Litter Quality and Earthworm Communities

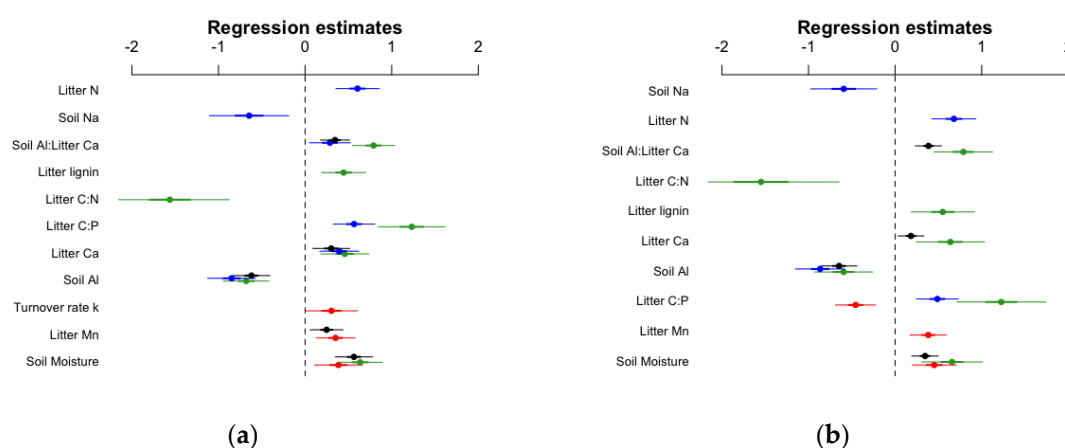
The earthworm groups were significantly linked with various soil and leaf litter characteristics (Tables 4 and 5; Figure 3). The best model fit was found for total and anecic earthworm density and biomass data. The models on endogeic and epigeic earthworm densities and biomasses showed a low increase in  $R^2$  (6%–14%) compared to the null model. In case of endogeic earthworm densities and biomasses, the final  $R^2$  was very low (21% and 28%) while these models contained seven selected predictor terms.

**Table 4.** Summary of the results identifying optimal models for total, anecic, endogeic and epigeic density and biomass. We used glmmADMB zero inflated models with Site as random effect, degrees of freedom (df), Akaike information criterion corrected for small sample sizes ( $AIC_c$ ) and  $R^2$  describing the correlation between fitted and observed values are shown. Soil predictor variables were measured in the topsoil (0–5 cm). Soil-Al:Litter-Ca is the interaction between exchangeable soil Al concentration and litter Ca concentration. The foliar litter nutrient concentrations were previously published by Vesterdal et al. [35,36].

Response Variable	Predictor Variables in Optimal Model	df	$AIC_c$ Optimal Model	$AIC_c$ Null Model	$R^2$ Optimal Model	$R^2$ Null Model
<i>Earthworm density</i>						
Total	Soil: Moisture, Al Litter: Ca, Mn Soil-Al:Litter-Ca	9	1061	1125	0.48	0.14
Anecic	Soil: Al, Na Litter: N, C:P ratio, Ca Soil-Al:Litter-Ca	10	766	850	0.62	0.12
Endogeic	Soil: Moisture, Al Litter: C:P ratio, C:N ratio, Ca, Lignin Soil-Al:Litter-Ca	11	725	800	0.28	0.14
Epigeic	Soil: Moisture Litter: Mn Forest floor turnover rate	7	804	817	0.42	0.36
<i>Earthworm biomass</i>						
Total	Soil: Moisture, Al Litter: Ca Soil-Al:Litter-Ca	7	821	902	0.53	0.12
Anecic	Soil: Al, Na Litter: N, C:P ratio	8	662	739	0.66	0.13
Endogeic	Soil: Al, Moisture Litter: Ca, Lignin, C:P ratio, C:N ratio Soil-Al:Litter-Ca	11	514	561	0.21	0.13
Epigeic	Soil: Moisture Litter: Mn, C:P ratio	7	482	509	0.53	0.40

**Table 5.** Summary of the results identifying optimal models for total, anecic, endogeic and epigeic density and biomass. We used glmmADMB zero inflated models with Site as random effect. The foliar litter nutrient concentrations were previously published by Vesterdal et al. [35,36].

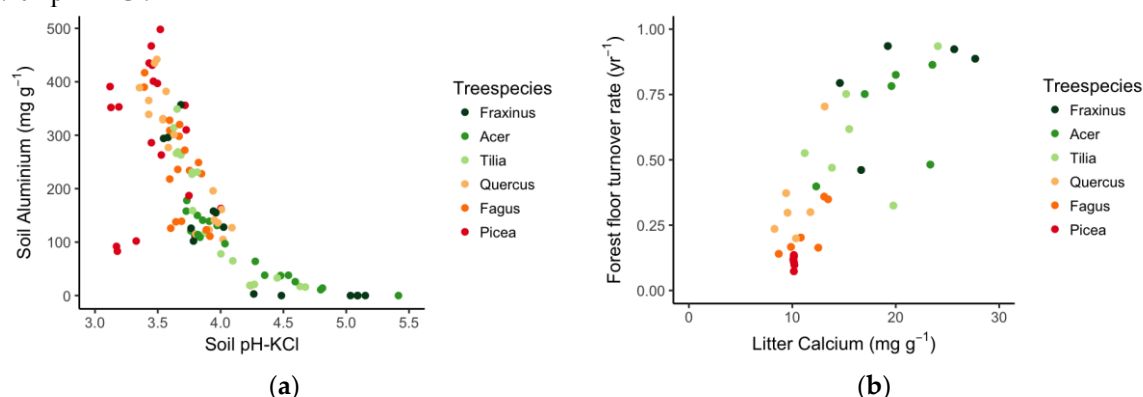
Predictor Variables	Total Density		Anecic Density		Endogeic Density		Epigeic Density	
	z value	p value	z value	p value	z value	p value	z value	p value
Soil Al	−5.7	<0.001	−6.0	<0.001	−5.1	<0.001		
Soil Na			−2.8	0.006				
Soil Moisture	5.1	<0.001			4.9	<0.001	2.7	0.007
Litter Ca	2.8	0.006	3.4	<0.001	3.2	0.001		
Litter N			4.7	<0.001				
Litter Mn	2.6	0.01					3.1	0.002
Litter lignin					3.4	<0.001		
Litter C:N ratio					−4.5	<0.001		
Litter C:P ratio			4.63	<0.001	6.2	<0.001		
Forest floor turnover rate							2.0	0.047
Soil-Al:Litter-Ca	4.0	<0.001	2.38	0.018	6.4	<0.001		
	Total Biomass		Anecic Biomass		Endogeic Biomass		Epigeic Biomass	
	z value	p value	z value	p value	z value	p value	z value	p value
Soil Al	−6.2	<0.001	−6.0	<0.001	−3.5	<0.001		
Soil Na			−3.1	0.002				
Soil Moisture	4.4	<0.001			3.7	<0.001	3.5	<0.001
Litter Ca	2.4	0.017			3.2	0.001		
Litter N			5.3	<0.001				
Litter Mn							3.6	<0.001
Litter lignin					3.0	0.003		
Litter C:N ratio					−3.4	<0.001		
Litter C:P ratio			4.0	<0.001	4.7	<0.001	−3.9	<0.001
Soil-Al:Litter-Ca	5.0	<0.001			4.6	<0.001		



**Figure 3.** Estimated effects of litter and soil (0–5 cm) variables on the earthworm density (a) and biomass (b). Predictor variables were standardized to the mean and scales by the standard deviation, so that effects correspond to a change in the earthworm density/biomass (on the log-scale) for a one standard deviation change in the predictor. This is true for all predictors, so their relative effects are comparable. The models are shown for total (black), anecic (blue), endogeic (green) and epigeic (red) earthworms. Coefficients are shown with confidence intervals by thick lines for 50% credible intervals and by thin lines for 95% credible intervals. We used glmmADMB zero inflated models with Site as group-level effect. The foliar litter nutrient concentrations were previously published by Vesterdal et al. [35,36].

Although the explanatory variables differed between the ecological earthworm groups, exchangeable soil Al and leaf litter Ca concentrations were almost always present in the final models for burrowing earthworms (i.e., anecic and endogeic species). Since pH-KCl seems to be better known

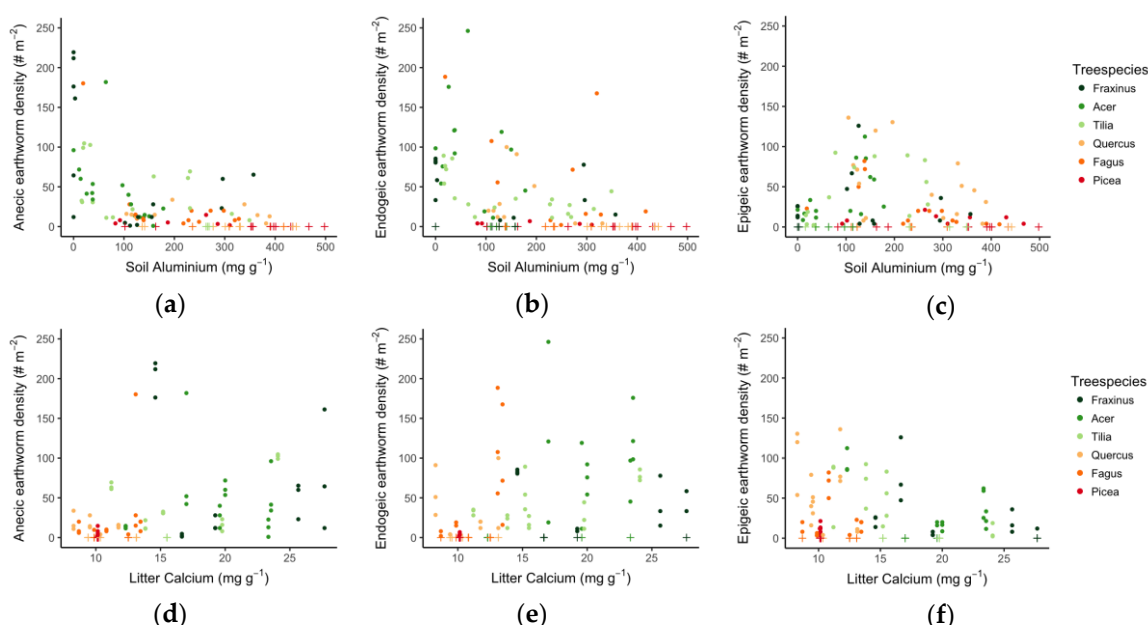
as a soil variable than exchangeable Al concentrations, we provide more insight into exchangeable Al in Figure 4a. Exchangeable Al concentrations are strongly inversely related with pH-KCl.



**Figure 4.** Relation between pH-KCl and exchangeable Al concentration in the 0–5 cm soil layer ((a)  $n = 105$ ). In (b), the relation between Ca concentration in foliar litter and the forest floor turnover rate ( $n = 35$ ) is shown. The points are colored according to the tree species. The foliar litter Ca concentration was previously published by Vesterdal et al. [36].

Burrowing earthworms related significantly negatively with soil Al concentrations, and were almost absent when soil pH-KCl decreased below a value of 4.0 (Figure 5a,b). Epigeic earthworms showed an optimum between pH-KCl 3.5 and 4.0 (Figure 5c).

Litter Ca concentrations were positively linked with forest floor turnover rates (Figure 4b) and burrowing earthworms (Figure 5d,e). Anecic and endogeic earthworm densities were generally not higher than 50 earthworms  $m^{-2}$  in stands with low litter Ca concentrations ( $<13 \text{ mg} \cdot g^{-1}$ ). Litter Ca concentration was not an explanatory variable for epigeic earthworms, which seems to be indifferent for this variable (Figure 5f).



**Figure 5.** Relation between exchangeable Al concentration in the 0–5 cm soil layer (a–c) or Ca concentration in foliar litter (d–f), and density of anecic (a,d), endogeic (b,e), and epigeic (c,f) earthworms. Plots where zero earthworms were found are indicated by a cross symbol. The foliar litter Ca concentration was previously published by Vesterdal et al. [36].

The interaction soil-Al:Litter-Ca represents a measure for the possible interaction between litter quality and soil quality affecting earthworm communities. The effect of litter Ca showed a more

positive effect on anecic earthworm density when soil Al concentrations were low. In contrast, this interaction shows a different pattern for endogeic density and biomass: the effect of litter Ca was more positive when soil Al concentrations were high. However, this effect was minor, as the models for endogeic earthworms had a low goodness of fit with the data.

Anecic earthworms were further significantly positively related with other litter quality variables such as N concentrations and C:P ratios. Additionally, lignin concentrations and C:N ratios were explanatory factors for the endogeic earthworms, although the fits were not very good. Finally, litter Mn concentrations and forest floor turnover rates were significant predictors for epigeic earthworms. Furthermore, both endogeic and epigeic earthworms were significantly positively linked with the soil moisture content.

To test whether different earthworm species of an ecological group had the same sensitivity towards soil Al and litter Ca concentrations, we selected the two most common earthworm species of each group (Appendix D). For the anecic species, *A. longa* was only scarcely present when soil Al concentrations were higher than  $50 \text{ mg}\cdot\text{g}^{-1}$  (and pH-KCl was below 4.2), while *L. terrestris* appeared to be abundantly present when litter with high Ca concentration was available. *A. longa* was the only earthworm species significantly associated with *Fraxinus*, *Acer* and *Tilia* (Appendix E). Similarly, but less pronounced was the difference between the two endogeic species: *A. rosea* seemed to be slightly more sensitive to high Al concentrations than *A. caliginosa* (Appendix D). The epigeic species, clearly present at a different pH-optimum (at pH-KCl 3.5–4.0), did not show a difference in sensitivity towards soil Al or litter Ca concentrations.

#### 4. Discussion and Conclusions

Within less than four decades, the observed tree species established specific diverging soil conditions and significantly affected the earthworm communities. Topsoils under *Picea*, *Fagus* and *Quercus* appeared to be, on average, four times more acid than under *Tilia*, *Acer* and *Fraxinus*, and this was independent of the soil type, land use history and climate. From literature, we already knew that intrinsic differences in leaf litter quality among tree species fundamentally create different soil conditions and nutrient cycling, both directly through the chemical composition of the litter, and indirectly through its effects on the size and composition of especially burrowing (endogeic and anecic) earthworm communities [25,27,31]. Tree species, such as *Picea*, *Fagus* and *Quercus*, with Ca-poor leaf litter, contribute to the absence of burrowing earthworm communities, which retards litter decomposition and results in forest floor build-up and high concentrations of exchangeable soil aluminium, which in turn negatively impacts on earthworm communities. Tree species with Ca-rich leaf litter such as *Fraxinus*, *Acer* and *Tilia* appeared to have abundant anecic and endogeic earthworm populations, lowest forest floor masses, highest forest floor turnover rates and highest pH values with lowest exchangeable Al concentrations. Other studies have reported good correlations between forest floor decomposition rates and earthworm populations, because of the positive influence of leaf litter quality on earthworm populations [26,27,31,33,54]. The fact that exchangeable Al concentrations are negatively influencing earthworm populations is less published.

When soils acidify below  $\text{pH}_{\text{H}_2\text{O}}$  of 5.0, base cations, such as Ca, are removed from the cation exchange complex and are replaced by Al [55]. The increase of exchangeable soil Al concentrations also takes place in the soil solution [55], which is toxic for earthworms [51]. High exchangeable Al concentrations and low pH values were found to inhibit earthworm growth and cocoon production [51]. Earthworms are negatively impacted by soil acidification and Ca-poor litter [27]. The absence of an abundant burrowing earthworm population decreases bioturbation and increases the build-up of a forest floor, which in turn delays cations becoming available again for buffering the proton input. This chain reaction and the complex interactions were well explained in the conceptual model in Figure 6 by De Schrijver et al. [27]. In our study, burrowing earthworm communities (endogeic and anecic species) appeared to be abundant when exchangeable soil Al concentrations were lower than  $100 \text{ mg}\cdot\text{Al}\cdot\text{g}^{-1}$ , and soil pH-KCl values were higher than about 4. Further soil acidification with exchangeable soil Al concentrations above  $100 \text{ mg}\cdot\text{Al}\cdot\text{g}^{-1}$  was associated with a complete absence of burrowing earthworms. However, favorable litter quality might compensate for unfavorable soil

conditions. Cesarz et al. [34] showed that an acid soil (pH-H<sub>2</sub>O 3.7–4.5, and probably high exchangeable Al concentrations) combined with Ca-rich leaf litter of *Tilia*, *Acer* or *Fraxinus* resulted in viable endogeic earthworm populations, while the combination of an acid soil with Ca-poor leaf litter of *Fagus* resulted in earthworm mortality.

In our study, we found a significant interaction between exchangeable Al concentrations and leaf litter Ca concentrations for anecic earthworm densities. The effect of high leaf litter Ca was more positive at low exchangeable Al concentrations. For endogeic earthworm density and biomass, the interaction was the other way around, but very weak. The significance of the interaction in our models indicates context-specificity by these plant–soil interactions: the effects of leaf litter Ca concentration on burrowing earthworms cannot be extrapolated from any one site (e.g., [31]) to other sites with different soil properties.

According to our models, epigeic earthworms were not significantly affected by both high soil Al concentrations or leaf litter Ca concentrations. Epigeic earthworm biomass appeared to be negatively linked with the C:P ratio in litter, which is in accordance with the findings of De Wandeler et al. (2016), and positively to soil moisture content and leaf litter Mn concentrations, which is most bioavailable in the pH-KCl-range of 3.4–4.1 [56].

*Picea* stands proved to be exceptionally unfavorable for earthworms from all three ecological groups. Next to an acidified topsoil and recalcitrant litter, having negative impacts on burrowing earthworm populations, these stands were also characterized by significantly lower soil moisture contents compared to the other tree species. Also, Christiansen et al. [56] showed consistently decreased soil moisture content in *Picea* stands compared to *Acer*, *Tilia*, *Fagus* and *Quercus* stands. In these drier conditions, epigeic earthworms, which were positively linked with soil moisture content according to our model, were almost absent. Epigeic species can endure short drought periods by producing drought-resistant cocoons, but when drought periods take too long they can go extinct locally [57]. The lower soil moisture content under *Picea* can contribute to low forest floor decomposition rates through its direct negative impact on the activity of soil biota [28].

Further, we found that soil salinization had taken place in the topsoil and deeper soil layers (15–30 cm) of *Picea* stands. Exchangeable soil Na concentrations were up to three times higher in the topsoil of *Picea* stands and were mainly increased in the sites enduring more westerly oceanic winds. These sites (Odsherred, Wedellsborg and Viemose) could be more influenced by marine sea salt deposition and *Picea* stands, known for their high atmospheric dry depositions [58] could, therefore, also capture more Na. Our models showed that anecic species were negatively related with soil Na concentration. In literature, the sensitivity of earthworms towards soil salinity was already reported for the endogeic *A. caliginosa* and the epigeic *Eisenia fetida* [59], but not for anecic species.

We combined existing data on various leaf litter quality characteristics and forest floor turnover rates with new data on soil biogeochemistry and earthworm communities to broaden the understanding of plant–soil interactions in this well-studied common garden experiment [35–37]. We found that total earthworm biomass in *Fraxinus*, *Acer* and *Tilia* stands was, on average, two times higher than in *Quercus* and *Fagus* stands and eight to ten times higher than in *Picea* stands. A total of 50% of the earthworm biomass in *Fraxinus*, *Acer* and *Tilia* stands was made up of two anecic species, namely *L. terrestris* and *A. longa*, which might explain the higher carbon stocks at 15–30 cm soil depth found by Vesterdal et al. [35]. Similar vertical distributions in soil C stock were found in stands of tree species from the same genera in other places, e.g., North America [10], suggesting specific tree species–soil interactions mediated by macrofauna species such as earthworms.

Within the endogeic and anecic ecological groups, a differentiation in sensitivity of earthworm species exists towards leaf litter quality (here illustrated by Ca concentration) and soil quality (here illustrated by exchangeable Al concentration). Because certain earthworm species can be bio-indicators for biological soil quality [21] and for forest site quality [17], studying the earthworm populations at species level can reveal greater detail in response to environmental conditions. The anecic *A. longa* and endogeic *A. rosea* appeared to be more sensitive to soil acidification (pH-KCl <4.0) than *L. terrestris* and *A. caliginosa*. Also, from our indicator species analyses, *A. longa* was associated with *Fraxinus*, *Acer* and *Tilia* stands. This is in accordance with the finding that *A. longa* is a sensitive

species towards pH that is closely associated with crop- and grasslands [60] and eutrophic deciduous forests [21]. *A. rosea*, however, was described previously as a species without a clear preference for a certain habitat type [21] and a tolerance for a broad pH-range [17,20]. In accordance with literature [21], the anecic *L. terrestris* and endogeic *A. caliginosa* appear to be species with a broad ecological niche because they were also found in soils with pH-KCl between 3.5 and 4.0. However, they seemed to appear in higher numbers when leaf litter contained more Ca. So, it seemed that the effects of high Al concentrations were mediated by nutrient-rich litter [34] for these two species. These findings imply the need to look beyond the response of rough earthworm ecological groups, and also focus on species-specific responses to varying leaf litter and soil characteristics.

In conclusion, we have shown that ecological earthworm groups are highly influenced by the tree species via several leaf litter and/or soil characteristics, but not all groups and species are affected similarly. According to Millennium Ecosystem Assessment [61], climatic change and atmospheric deposition of reactive N, are two of the major drivers of biodiversity loss in forests. Future climate change may come in the form of higher summer temperatures and/or increased droughts in temperate European regions [62]. On the other hand, atmospheric N deposition is expected to rise even further in temperate European regions and cause acidification [61]. Climate change may even worsen the effects of acidification by air pollution [63]. Future studies of plant–soil interactions should consider how the magnitude of litter impacts on soil organisms and soil processes might depend on how bedrock, climate, and atmospheric pollution have influenced soil acidification. Our results have shown that endogeic and epigeic earthworms were sensitive to drought, and endogeic and anecic earthworms were sensitive to acidification. In our study, we saw that planting *Picea* on these soils that are prone to acidification, resulted in an almost complete eradication of the earthworm population. To avoid the loss of earthworm biodiversity and functioning, foresters can mitigate these expected global changes by a substantiated choice of tree species.

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**Author Contributions:** L.V. and A.D.S. conceived and designed the study; S.S., L.V. and A.D.S. carried out the fieldwork; S.S., A.D.S., L.B. and J.M. analyzed the data; S.S., A.D.S., J.M., L.V., K.V. and B.M. wrote the manuscript.

**Conflicts of interest:** The authors declare no conflict of interest.

## Appendix A

**Table A1.** Longitude and latitude for each site.

Site	Longitude, Latitude
Kragelund	56°10' N, 9°25' E
Mattrup	55°57' N, 9°38' E
Odsherred	55°50' N, 11°42' E
Vallø	55°25' N, 12°03' E
Viemose	55°01' N, 12°09' E
Wedellsborg	55°24' N, 9°52' E

## Appendix B

**Table A2.** Details of the results identifying optimal models for total, anecic, endogeic and epigeic density and biomass. We used glmmADMB zero inflated models with Site as random effect. Soil predictor variables were measured in the topsoil (0–5 cm). Soil-Al:Litter-Ca is the interaction between exchangeable soil Al concentration and litter Ca concentration. The foliar litter nutrient concentrations were previously published by Vesterdal et al. [35,36].

Response Variable	Predictor Variables in Model	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	Weight
<i>Earthworm density</i>				
Total	null model	1125	64	<0.001
	Soil-Al	1072	11	0.002
	Soil-Al + Litter-Ca	1074	13	<0.001
	Soil-Al + Litter-Ca + Soil-Al:Litter-Ca	1064	4	0.11
	Soil-Al + Litter-Ca + Soil-Al:Litter-Ca + Litter-Mn	1063	2	0.25
	Soil-Al + Litter-Ca + Soil-Al:Litter-Ca + Litter-Mn + Soil Moisture	1061	0	0.64
Anecic	null model	850	84	<0.001
	Soil-Al	787	21	<0.001
	Soil-Al + Litter-N	786	20	<0.001
	Soil-Al + Litter-N + Litter-C:P	778	13	0.0014
	Soil-Al + Litter-N + Litter-C:P + Soil-Na	770	5	0.08
	Soil-Al + Litter-N + Litter-C:P + Soil-Na + Litter-Ca	769	3	0.17
	Soil-Al + Litter-N + Litter-C:P + Soil-Na + Litter-Ca + Soil-Al:Litter-Ca	766	0	0.75
Endogeic	null model	800	75	<0.001
	Soil-Al	788	63	<0.001
	Soil-Al + Soil-Al:Litter-Ca	784	60	<0.001
	Soil-Al + Soil-Al:Litter-Ca + Litter-Ca	781	57	<0.001
	Soil-Al + Soil-Al:Litter-Ca + Litter-Ca + Soil Moisture	750	26	<0.001
	Soil-Al + Litter-Ca + Soil-Al:Litter-Ca + Soil Moisture + Litter-C:P	740	14	<0.001
	Soil-Al + Litter-Ca + Soil-Al:Litter-Ca + Soil Moisture + Litter-C:P + Litter-C:N	735	10	0.0058
	Soil-Al + Litter-Ca + Soil-Al:Litter-Ca + Soil Moisture + Litter-C:P + Litter-C:N + Litter-lignin	725	0	0.99
Epigeic	null model	817	13	0.0012
	Soil Moisture	810	6	0.036
	Soil Moisture + Litter-Mn	806	2	0.28
	Soil Moisture + Litter-Mn + Forest floor turnover rate	804	0	0.68
<i>Earthworm biomass</i>				
Total	null model	902	79	<0.001
	Litter-Ca	874	52	<0.001
	Litter-Ca + Soil-Al:Litter-Ca	869	47	<0.001
	Litter-Ca + Soil-Al:Litter-Ca + Soil-Al	833	10	0.006
	Litter-Ca + Soil-Al:Litter-Ca + Soil-Al + Soil Moisture	822	0	0.99
Anecic	null model	739	77	<0.001
	Soil-Al	683	20	<0.001
	Soil-Al + Litter-N	676	13	0.0013
	Soil-Al + Litter-N + Litter-C:P	670	8	0.020
	Soil-Al + Litter-N + Litter-C:P + Soil-Na	662	0	0.98
Endogeic	null model	561	47	<0.001
	Soil-Al	540	26	<0.001
	Soil-Al + Soil-Al:Litter-Ca	532	19	<0.001
	Soil-Al + Soil-Al:Litter-Ca + Litter-Ca	529	16	<0.001
	Soil-Al + Soil-Al:Litter-Ca + Litter-Ca + Soil Moisture	525	12	0.0022
	Soil-Al + Litter-Ca + Soil-Al:Litter-Ca + Soil Moisture + Litter-C:P	517	4	0.10
	Soil-Al + Litter-Ca + Soil-Al:Litter-Ca + Soil Moisture + Litter-C:P + Litter-C:N	516	2	0.22
	Soil-Al + Litter-Ca + Soil-Al:Litter-Ca + Soil Moisture + Litter-C:P + Litter-C:N + Litter-lignin	514	0	0.67
Epigeic	null model	509	27	<0.001
	Soil Moisture	498	16	<0.001
	Soil Moisture + Litter-Mn	496	14	0.0012
	Soil Moisture + Litter-Mn + Litter-C:P	482	0	1.00

## Appendix C

**Table A3.** Mean and standard deviation of the deeper soil (5–15 cm) properties for each tree species across all six common gardens. Significant differences according to the Tukey post-hoc test between tree species are indicated with letters, means with the same letter are not significantly different (Tukey post-hoc tests on LME models, 1|Site).

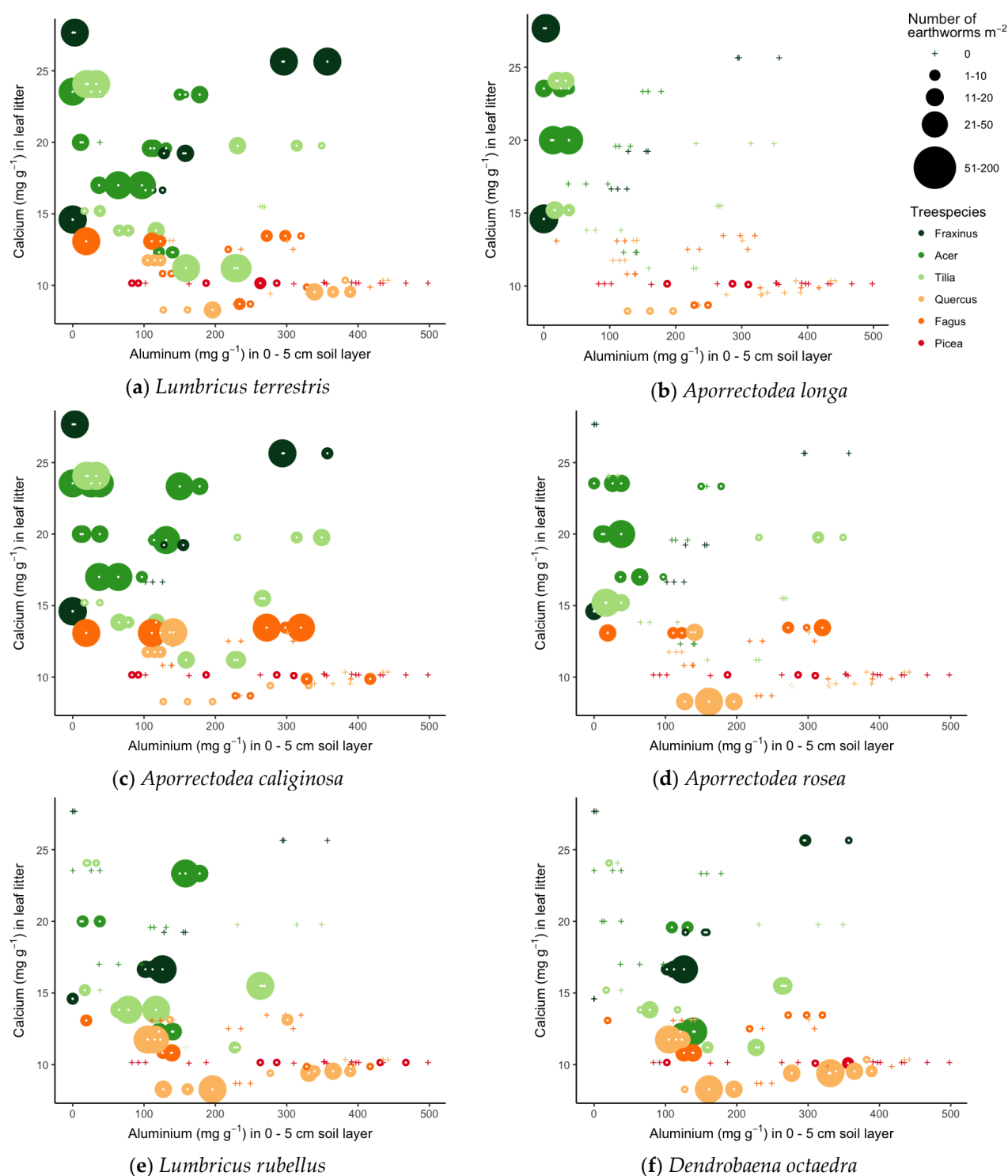
Soil variables (15–30 cm)	f-value	p	Tree Species					
			<i>Fraxinus</i>	<i>Acer</i>	<i>Tilia</i>	<i>Quercus</i>	<i>Fagus</i>	<i>Picea</i>
pH-KCl	275	<0.001	4.2 ± 0.58 <sup>c</sup>	4 ± 0.37 <sup>bc</sup>	3.9 ± 0.28 <sup>ab</sup>	3.8 ± 0.27 <sup>a</sup>	3.8 ± 0.17 <sup>a</sup>	3.7 ± 0.26 <sup>a</sup>
Base saturation (%)	10	<0.001	60 ± 36 <sup>bc</sup>	60 ± 30 <sup>c</sup>	43 ± 27 <sup>ab</sup>	35 ± 28 <sup>a</sup>	36 ± 26 <sup>a</sup>	41 ± 32 <sup>a</sup>
K in BaCl <sub>2</sub> (mg·K·g <sup>-1</sup> )	28	<0.001	38 ± 17 <sup>b</sup>	54 ± 57 <sup>b</sup>	43 ± 35 <sup>b</sup>	46 ± 34 <sup>b</sup>	36 ± 23 <sup>b</sup>	28 ± 20 <sup>a</sup>
Na in BaCl <sub>2</sub> (mg·Na·g <sup>-1</sup> )	26	<0.001	17 ± 19 <sup>a</sup>	13 ± 9 <sup>a</sup>	10 ± 5 <sup>a</sup>	9 ± 7 <sup>a</sup>	11 ± 6 <sup>a</sup>	37 ± 47 <sup>b</sup>
Mg in BaCl <sub>2</sub> (mg·Mg·g <sup>-1</sup> )	16	<0.001	77 ± 81 <sup>b</sup>	61 ± 61 <sup>b</sup>	33 ± 26 <sup>a</sup>	38 ± 42 <sup>ab</sup>	29 ± 25 <sup>a</sup>	53 ± 45 <sup>ab</sup>
Ca in BaCl <sub>2</sub> (mg·Ca·g <sup>-1</sup> )	18	<0.001	954 ± 1049 <sup>b</sup>	659 ± 603 <sup>b</sup>	375 ± 358 <sup>ab</sup>	357 ± 433 <sup>a</sup>	312 ± 288 <sup>a</sup>	482 ± 481 <sup>a</sup>
Al in BaCl <sub>2</sub> (mg·Al·g <sup>-1</sup> )	57	<0.001	133 ± 131 <sup>a</sup>	136 ± 87 <sup>ab</sup>	211 ± 118 <sup>bc</sup>	262 ± 133 <sup>c</sup>	232 ± 103 <sup>bc</sup>	248 ± 136 <sup>bc</sup>

**Table A4.** Mean and standard deviation of the deeper soil (15–30 cm) properties for each tree species across all six common gardens. Significant differences according to the Tukey post-hoc test between tree species are indicated with letters, means with the same letter are not significantly different (Tukey post-hoc tests on LME models, 1|Site).

Soil variables (15–30 cm)	f-value	p	Tree Species					
			<i>Fraxinus</i>	<i>Acer</i>	<i>Tilia</i>	<i>Quercus</i>	<i>Fagus</i>	<i>Picea</i>
pH-KCl	236	<0.001	4.4 ± 0.57 <sup>b</sup>	4.2 ± 0.37 <sup>ab</sup>	4.0 ± 0.31 <sup>a</sup>	4.1 ± 0.4 <sup>ab</sup>	4.1 ± 0.43 <sup>ab</sup>	4.1 ± 0.37 <sup>ab</sup>
Base saturation (%)	4.0	<0.005	60 ± 38	55 ± 33	39 ± 33	46 ± 33	51 ± 35	51 ± 39
K in BaCl <sub>2</sub> (mg·K·g <sup>-1</sup> )	13	<0.001	29 ± 21	32 ± 38	29 ± 26	34 ± 29	27 ± 24	26 ± 22
Na in BaCl <sub>2</sub> (mg·Na·g <sup>-1</sup> )	17	<0.001	17 ± 20 <sup>a</sup>	12 ± 7 <sup>a</sup>	9,0 ± 5,4 <sup>a</sup>	10 ± 9,5 <sup>a</sup>	13 ± 8,7 <sup>a</sup>	42 ± 58 <sup>b</sup>
Mg in BaCl <sub>2</sub> (mg·Mg·g <sup>-1</sup> )	8.7	<0.001	76 ± 94 <sup>b</sup>	47 ± 59 <sup>ab</sup>	28 ± 33 <sup>a</sup>	51 ± 60 <sup>ab</sup>	43 ± 44 <sup>ab</sup>	58 ± 55 <sup>ab</sup>
Ca in BaCl <sub>2</sub> (mg·Ca·g <sup>-1</sup> )	13	<0.001	1109 ± 1252 <sup>b</sup>	590 ± 694 <sup>ab</sup>	339 ± 402 <sup>a</sup>	522 ± 628 <sup>a</sup>	527 ± 524 <sup>ab</sup>	692 ± 733 <sup>ab</sup>
Al in BaCl <sub>2</sub> (mg·Al·g <sup>-1</sup> )	37	<0.001	115 ± 113	128 ± 86	175 ± 105	175 ± 117	149 ± 97	149 ± 113



## Appendix D



**Figure A1.** The density of the most common earthworm species (anecic: *L. terrestris* (a) and *A. longa* (b); endogeic: *A. caliginosa* (c); *A. rosea* (d); and epigeic: *L. rubellus* (e) and *D. octaedra* (f) in relation with exchangeable soil Al concentration and Ca concentration in litter. Earthworm density is shown by the size of the circles; a cross symbol indicates plots where no earthworms were found. The color of the circle indicates the tree species. The foliar litter Ca concentration was previously published by Vesterdal et al. [36].

## Appendix E

The dataset was screened for associations between all earthworm species (density and biomass) and the six tree species by indicator species analysis [64] allowing for the combination of tree species [65] by using the function *multipatt* (package *indicpecies* [66]) with “*IndVal*” as the statistical index with 999 permutations. The calculated Indicator Value is the product of two components: a specificity component (A) and a sensitivity component (B) of earthworm species for a tree species.

**Table A5.** Indicator species analysis of earthworm density and biomass. The indicator component A (specificity) relates to the probability that the earthworm species only occurs in this group of tree species. The indicator component B (sensitivity) relates to the probability of finding the earthworm species in this group of tree species.

	Earthworm Species	Component A	Component B	IndVal	p-Value
Association with tree species group:					
<b>Earthworm density</b>					
<i>Fraxinus</i> + <i>Acer</i> + <i>Tilia</i>	<i>A. longa</i>	0.97	0.33	0.57	0.008
<i>Fraxinus</i> + <i>Acer</i> + <i>Tilia</i> + <i>Quercus</i>	<i>L. rubellus</i>	0.92	0.59	0.74	0.009
<i>Fraxinus</i> + <i>Acer</i> + <i>Tilia</i> + <i>Fagus</i>	<i>L. terrestris</i>	0.92	0.87	0.89	0.001
<i>Fraxinus</i> + <i>Acer</i> + <i>Tilia</i> + <i>Quercus</i> + <i>Fagus</i>	<i>A. caliginosa</i>	0.99	0.70	0.84	0.001
	<i>D. octaedra</i>	0.98	0.53	0.72	0.006
<b>Earthworm biomass</b>					
<i>Fraxinus</i> + <i>Acer</i> + <i>Tilia</i>	<i>A. longa</i>	0.98	0.33	0.57	0.014
<i>Fraxinus</i> + <i>Acer</i> + <i>Tilia</i> + <i>Quercus</i>	<i>L. rubellus</i>	0.92	0.59	0.74	0.006
<i>Fraxinus</i> + <i>Acer</i> + <i>Tilia</i> + <i>Quercus</i> + <i>Fagus</i>	<i>L. terrestris</i>	0.97	0.82	0.89	0.001
	<i>A. caliginosa</i>	0.99	0.70	0.83	0.002
	<i>D. octaedra</i>	0.96	0.53	0.71	0.010

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